Naval Research Laboratory
Washington, DC 20375-5320

NRL/MR/6110--10-9258

Microbial Gas Production Used to Achieve Autonomous Buoyancy Control

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September 1, 2010

135671

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-09-2010	NRL Memorandum Report	

4. TITLE AND SUBTITLE

Microbial Gas Production Used to Achieve Autonomous Buoyancy Control

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
61-6119-A-0

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Research Laboratory, Code 6113
4555 Overlook Avenue, SW
Washington, DC 20375-5320

8. PERFORMING ORGANIZATION REPOR NUMBER

NRL/MR/6110--10-9258

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

10. SPONSOR / MONITOR'S ACRONYM(S

11. SPONSOR / MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have constructed and successfully laboratory tested a device that can periodically change from a submerged to a buoyant state usi gas generated by microbes alone. The duration of the buoyant state and the switching frequency from the submerged to buoyant state can controlled. If the type of microbes used is native to the deployment location, gas generation will not create an identifiable acoustic signature disclose the presence and operation of the device. In this report, we discuss the operation and testing of the device, the microbes that we us to test the operation of the device, and the test results.

15. SUBJECT TERMS

Distributed autonomous sensors Microbial gas generation
Ballast control

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PER
					Bradley R. Ringeisen
a. REPORT	b. ABSTRACT	c. THIS PAGE	SAR	12	19b. TELEPHONE NUMBER *(include code)*
Unclassified	Unclassified	Unclassified			(202) 767-0719

Microbial Gas Production Used to Achieve Autonomous Buoyancy Control

Introduction

A field deployed device designed to operate autonomously for long periods (years) of time requires special designs to maximize its operational lifetime. One strategy is to design such a device to take advantage of the ambient resources and conditions at the point of deployment. For example, clandestine submerged devices for underwater surveillance should be designed to exploit the marine resources to maximize its operation duration and minimize its size and weight.

Distributed autonomous sensor networks equipped with acoustic or magnetic sensors may soon be used to detect and track submarines in the littoral regions of the ocean. In order to maximize the effectiveness of the sensor network, power is required to surface each sensor periodically so that accumulated data can be communicated via RF or UHF transmission and the position of the sensor can be determined (communication with global positioning systems). These transmissions are impossible for submerged devices, as radio frequencies do not propagate well underwater.

Due to modern advances in electronics, which have reduced the power consumption in circuitry, aquatic persistent surveillance devices may be powered by microbial fuel cells in the foreseeable future [1-3]. Electric generation using microbes shows promise for devices that are designed for long term deployment without servicing. Moreover, since many microbes are capable of survival in dark environments, the use of microbes for electric generation where solar power is not an option (underwater applications) is especially promising. The utilization of nutrients from marine environments by microbes could potentially extend the operation of the circuitry in the device indefinitely.

The ability of a submerged device to surface periodically posts a different challenge. For a device with a constant mass, to convert from a submerged to a buoyant state, the displacement of the device must change. The most common strategy is to induce a change in displacement of the device by using various methods to push water from the device, such as pumps or compressed gas. The submerged and buoyant state can be switched using multiple valves. Such a scheme requires a source of compressed gas or a pump and additional power to operate the valves and timing circuits. This increases the size, weight, and acoustic signature of the device.

Manuscript approved March 24, 2010.

The operational lifespan of the device is ultimately limited by the amount of compressed gas stored or by the battery life to power the pumps and valves.

We have constructed and successfully laboratory tested a device that can periodically change from a submerged to a buoyant state using gas generated by microbes alone. The duration of the buoyant state and the switching frequency from the submerged to buoyant state can be controlled. If the type of microbes used is native to the deployment location, gas generation will not create an identifiable acoustic signature to disclose the presence and operation of the device. In this report, we will discuss the operation and testing of the device, the microbes that we used to test the operation of the device, and the test results.

General Description of the Periodically-Surfacing Submerged-Device

The proposed device consists of the following parts, see Figure 1: a chamber, float, and a counter weight. We will first describe the function and design of each component.

1. The device chamber:

The device chamber can be of a wide variety of shapes. For the purpose of illustrating the operation of the device, we have chosen to use a simple closed tube with two holes. For different device chamber shapes and sizes, the weight and location of the other parts of the device must be adjusted to accommodate the operation of the device.

To keep the description of the operation of the device simple, we will assume that the device chamber is neutrally buoyant in the fluid under consideration. This means that no additional force is added to the device chamber by its own weight. This can be achieved in reality by attaching weight or floatation material to the device chamber to achieve a neutrally buoyant condition. In other words, there is no

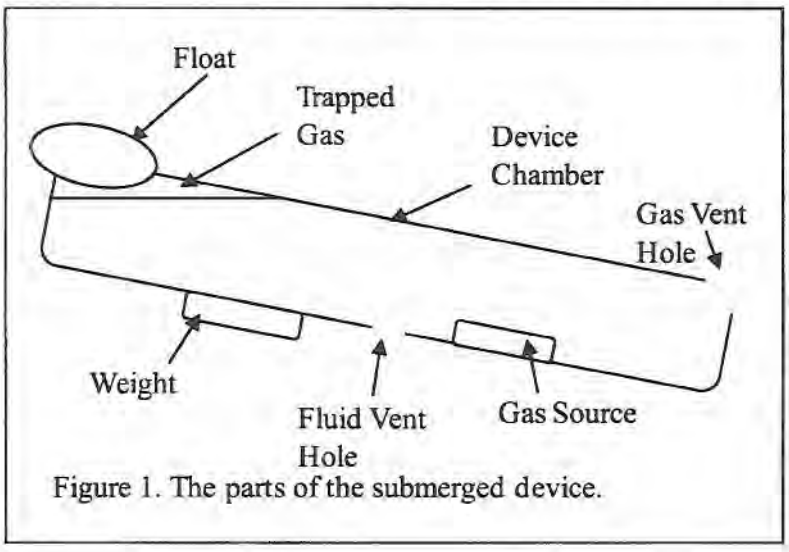

Figure 1. The parts of the submerged device.

limitation to the weight, size, and shape of the chamber.

2. The Float

A float of lower density than the fluid is attached to the device chamber. It can be physically attached or linked to the chamber with a flexible cable, chain, string, or connection of any kind. The anchoring point should be located toward one end of the device chamber and most convenient on the top side of the device chamber. The float provides a buoyant force, F_f, on one end of the device chamber. The amount of buoyant force required and the exact anchoring location of the float (where the buoyant force of the float acts on the device chamber) are design parameters for customizing the operation of the device.

In the orientation shown in Figure 1, gas produced from the gas source is trapped within the device chamber. This is an essential part of the design. The device will float because of the introduction of gas and thus increase in displacement. The trapped gas will produce a buoyant force, F_b, on the device chamber. The location of the line-of-action of the buoyant force is a function of the amount of trapped gas as well as the shape, weight, and location of all the other components in the device. As gas is introduced into the chamber, the fluid must be expelled. A fluid vent hole is located on the bottom side of the device chamber for the displaced fluid to escape.

The operation of this device requires a source of gas. The source of gas will be products of metabolism of the microbes. The gas source can be attached to the device chamber in any convenient location and by any convenient method but the gas must become trapped in the chamber.

For the device to transition to a submerged state, the trapped gas must be removed. A gas vent hole is located on the top side of the device chamber and on the side of the device chamber opposite to the attachment point of the float.

3. The Counter Weight

A weight of higher density than the fluid is used to create a downward force to the device. For convenience of demonstration here, it is attached to the bottom of the device chamber. In a real device, the weight can be designed to be attached in different locations. It can be part or all of the device chamber and/or the gas source. It can be physically attached or

linked to the chamber with a flexible cable, chain, or string. For simplicity of description, we showed here the case where the anchoring point is located closer to the float attachment point than the gas vent hole. The weight provides a downward force, F_w, on the device chamber. The amount of downward force required and the exact anchoring location of the weight (where the downward force of the weight acts on the device chamber) are design parameters for customizing the operation of the device.

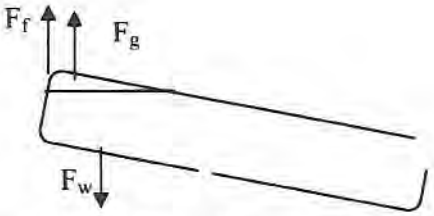

Figure 2. Free-body-diagram of the proposed device with neutrally buoyant chamber and gas source.

No payload is specified here, but it can be part or all of the device chamber, float, weight, or the gas source. It can also be a separate unit attached directly or through a cable, string, chain, or any connector. It can be of any size, shape, and weight. In the discussion below, we can see that none of these attributes are a limiting factor to the operation of the device.

The Operation of the Device:

The total force acting on the device consists of three forces: the buoyant forces from the float, F_f, the trapped gas, F_g, and the weight, F_w. No other forces are present because we made the physically achievable condition that the device chamber and the gas source are neutrally buoyant. A simple free-body-diagram can be drawn and is shown in Figure 2.

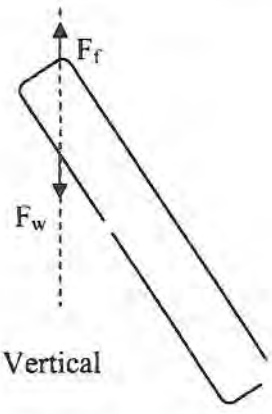

Figure 3. Initial equilibrium condition: only two forces are present and they must lie on a vertical line.

To understand the operation of the device, we will examine the behavior of the device chamber as gas is being introduced to the chamber starting from the condition of no trapped gas. In each case, we will examine the free-body diagram to determine the

behavior of the device. We require the magnitude of the counter weight to be larger than that of the buoyant force, $|F_w| > |F_f|$. This means that without the presence of trapped gas, the device chamber will sink.

1. Initial condition - no trapped gas

Because there is no trapped gas, i.e., $F_g = 0$, the device chamber will rotate until the direction of F_w and F_f are opposite to each other in the vertical position, Figure 3. Because of the location of the anchoring points of the float and the weight, the device chamber is tilted to one side with the gas vent hole rotated toward the bottom of the float chamber. Because $|F_w| > |F_f|$, the device has a net force pointing down and will sink.

Any gas supplied by the gas source will be trapped in the top closed end of the device. The presence of gas will have two effects on the device chamber: First, the net force pointing down will be reduced because F_b should always point up. Second, because of the asymmetry of the trapped gas volume, the device will rotate to achieve a zero net torque.

2. Some trapped gas but the net force still points down.

As gas enters the device chamber, a buoyant force, F_g, is introduced. The line-of-action for F_g, should be pointing upward and through the center of gravity of the trapped gas volume. The free body diagram is shown in Figure 4.

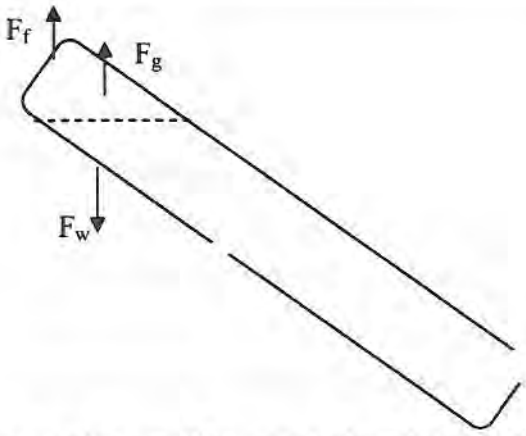

Figure 4. After a small amount of gas is trapped in the device chamber, the net downward force is reduced and the device chamber is rotated.

The resultant of the three forces is a reduction in the net downward force and a rotation of the device chamber because of the asymmetry in the trapped gas volume. The device will remain submerged as long as $F_w > F_g + F_f$.

3. More gas is trapped and $F_w = F_g + F_f$, but the device chamber is still tilted down to the right.

At this point the whole device is neutrally buoyant and further introduction of gas will result in a net force pointing up. The device will begin to rise as more gas is trapped. The location of the anchors for the float and weight are chosen such that the gas vent hole is still on the down side of the device chamber.

The time it takes for the device chamber to reach this condition depends on the rate of gas input, the shape and size of the device chamber, and the magnitude and location of F_w and F_f. These and other factors determine the total submerged time of the device.

4. More gas is trapped and F_g continue to move away from F_f. At some point the torque produced by F_g is greater than that of F_f, the device chamber will start to rotate such that the right side of the chamber is pointing up.

As the device chamber starts to rotate, the center of gravity of the trapped gas will flow further away from F_f, further increasing the torque by F_g. This is a positive feedback situation; the rotation will accelerate until the device chamber is oriented similar to that shown in Figure 5. This orientation of the device chamber, however, is not a stable state. Because the gas vent hole is now on top, the gas will vent out of the device chamber. As the gas leaks out, the magnitude of F_g will start to decrease.

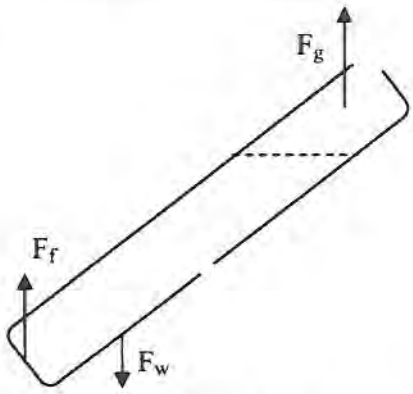

Figure 5. A large amount of trapped gas will rotate the device chamber. It will achieve a new orientation and the gas will vent out of the gas vent hole in this orientation. This is not a stable state condition.

As F_g decreases, the net force pointing up and the torque to keep the device chamber in the orientation shown in Figure 5 will decrease. Eventually, enough gas will leak out and the net force will point down, $F_w > F_g + F_f$, such that the device chamber will start to sink. The torque from F_f will decrease and the device chamber will eventually return to the condition shown in Figure 1 or 2 depending on whether all or part of the gas has time to vent out of the device chamber.

Note that it is possible that the float chamber never surfaces. This situation can be useful in some applications. For example, if the payload is the float and is tattered to the device chamber, the device will start to rise when $F_w < F_g + F_f$. The rise will continue until the payload reaches the surface. After that, the device chamber will remain submerged. This is possible because, at this point, the buoyant force of the gas, F_g, is the only upward force (the float surfaced and $F_f = 0$) and it may be weaker than the weight, F_w. In this case, the device chamber remains submerged after the payload surfaces. Depending on the specific design, the float chamber can start to tilt before it ever reaches the surface. But it does not matter because the payload does surface.

The process, step1 through 4 will repeat itself as long as gas is continuously produced and introduced into the device chamber.

To test the operation of this device, we built a device chamber using a 50 mL Falcon tube, a fishing bobbin as a float and an Erlenmeyer flask with added weight as a counter weight. The microbe used is cultured in the Erlenmeyer flask.

Clostridium acetobutylicum for microbial ballast

In order to utilize microbial ballast for the periodic surfacing of a submerged device, the microbe must be able to produce gas under anaerobic conditions. For this reason we chose to use *Clostridium acetobutylicum* as our model microbe; a gram-positive anaerobic bacterium known for its ability to produce hydrogen gas [4,5].

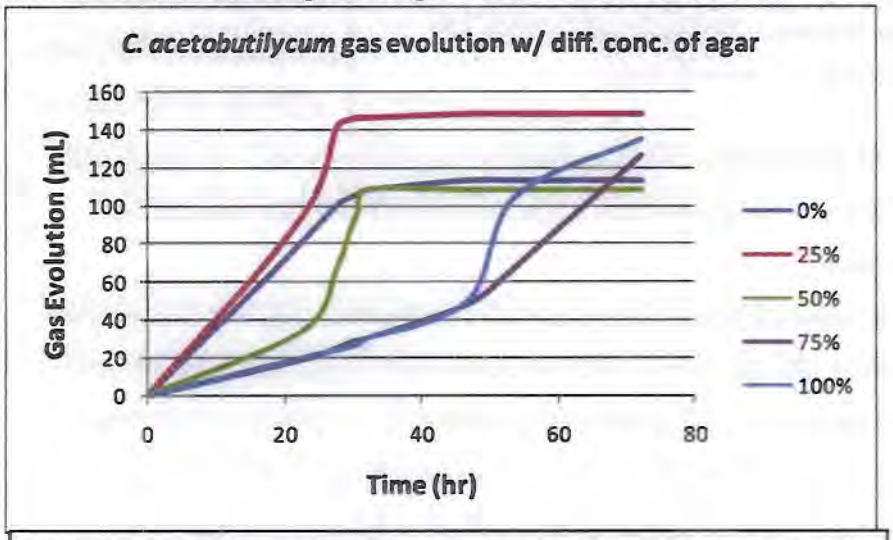

Figure 6. Gas production by different solid-phase agar cultures of C. acetobutylicum.

To determine if there was a difference in gas production in solid vs. liquid mediums, we cultured *C. acetobutylicum* in reinforced clostridal medium (OXOID

CM0149) with four different concentrations of agar (we defined 100% agar as the 'typical' agar concentration in a solid medium support (1.5g/100mL) and therefore 75%, 50%, and 25% agar concentrations refer to a final agar concentration of 1.12, 0.75, and 0.38g of agar per 100mL, respectively) or no agar at all. Our results (Figure 6) conclude that *C. acetobutylicum* has the highest gas production when cultured in reinforced clostridal medium with 25% agar as the solid support. However, our results also indicate that after ~28 hrs there was a cessation of gas evolution; most likely due to the depletion of carbon food sources.

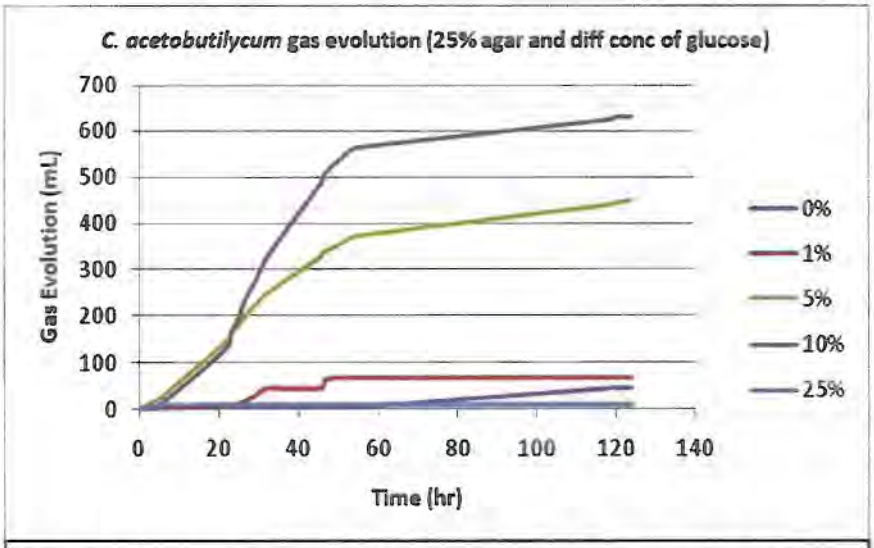

Figure 7. Gas production by different solid-phase agar cultures of C. acetobutylicum with varying glucose concentration.

For the continuation of gas evolution for sustained amounts of time, a food source must be present in the medium. For this study, glucose was chosen as the carbon source. A culture was grown on 25% agar reinforced clostrium medium with different concentrations of glucose (1, 5, 10, or 25g of glucose per 100mL culture) or no glucose at all. The production of gas was extended from 28 hrs to 50 hrs before it began to level off and then discontinue production after 120 hrs (Figure 7).

Future studies will incorporate time-released capsules within the medium to slow down the initial evolution of gas from 180 mL/day to 10-20 mL/day which would allow for the device to submerge and surface 2-3 times a day using our testing apparatus. Furthermore, the time-released capsules of glucose would not only slow down the gas production but it will also provide *C. acetobutylicum* with a constant supply of food source to sustain long deployment times

Test Apparatus

As described earlier, the device chamber used is a 50 mL Falcon tube (see Figure 8). Two 6 mm diameter holes were drilled in the Falcon tube. The gas vent hole was drilled at the tapered end of the Falcon tube. The fluid vent hole was drilled on the side of the tube approximately in the middle.

A common fishing bobbin, available in any fishing supply store, was used as the float. The float was attached to the cap of the Falcon tube with electrician tape via a ~ 5 mm long wire fishing line and a swivel. This allowed the float to rotate freely independent of the orientation of the device chamber.

Figure 8. Fully assembled ballast control device with ballast chamber and solid-phase *C. acetobutylicum* culture.

The Erlenmeyer flask containing a *C. acetobutylicum* solid-phase culture was attached to the device chamber via a fishing line and a swivel so it could rotate independently of the orientation of chamber. The gas from the flask was introduced into the chamber through a plastic tube through the fluid vent hole. The tube from the flask was first bent into a loop before it entered the device chamber. This loop acted as a gas lock so that outside gas or fluid could not flow into the Erlenmeyer flask but excess gas could escape from the flask. The apparatus was tested in a 50 gallon water tank, where it surfaced and re-submerged every 30 minutes for 24 hours without any input energy beyond the chemical food supplied to the microorganisms.

Conclusions

We have demonstrated a zero-power ballast control system that could be used to reduce the total power budget of underwater distributed autonomous sensors. The method utilizes microbial gas generation coupled to a ballast chamber to periodically float and re-submerge a test buoy. Future experiments will slow the production of gas via controlled-release of nutrients to the microorganisms so that fewer floating and re-submersion cycles will occur, lengthening the functionality of the ballast control from a few days to a few months. Ultimately, the device will

utilize nutrients scavenged from the natural littoral environment so that gas generation can be sustained for years.

References

(1)Tender, L. M.; Gray, S. A.; Groveman, E.; Lowy, D. A.; Kauffman, P.; Melhado, J.; Tyce, R. C.; Flynn, D.; Petrecca, R.; Dobarro, J. *J. Power Sources* **2008**, *179*, 571-575.
(2)Bond, D. R.; Holmes, D. E.; Tender, L. M.; Lovley, D. R. *Science* **2002**, *295*, 483-485.
(3)Ringeisen, B. R.; Henderson, E.; Wu, P. K.; Pietron, J.; Ray, R.; Little, B.; Biffinger, J. C.; Jones-Meehan, J. M. *Environ. Sci. Technol.* **2006**, *40*, 2629-2634.
(4)Argun, H.; Kargi, F.; Kapdan, I. K. *Int. J. Hydrogen Energy* **2009**, *34*, 2195-2200.
(5)Zhang, H.; Bruns, M. A.; Logan, B. E. *Water Res.* **2006**, *40*, 728-734.